Induction of early neural precursors and derivation of tripotent neural stem cells from human pluripotent stem cells under xeno-free conditions.

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Public Summary:
Human embryonic stem cells (hESC) and induced pluripotent stem cells (hiPSC) can differentiate into many cell types and are important for regenerative medicine; however, further work is needed to reliably differentiate, or turn hESC and hiPSC into neural-restricted multipotent derivatives capable of making the specialized cell types of the brain and CNS and not other cell types. Further, procedures are needed to enable us to make neural stem cells under conditions that are free from animal products so that they might be applicable for translational work aimed towards human therapies. We tested the transition of hESC and hiPSC lines onto xeno-free (XF) / feeder-free conditions and evaluated XF substrate preference, pluripotency, and karyotype. Critically, XF transitioned H9 hESC, Shef4 hESC, and iPS6-9 retained pluripotency, proliferation, and normal karyotype. Subsequently, XF transitioned hESC and hiPSC were induced with epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) to generate neuralized spheres containing primitive neural precursors, which could differentiate into astrocytes and neurons, but not oligoprogenitors. Further neuralization of spheres via LIF supplementation and attachment selection on CELLstart substrate generated adherent human neural stem cells (hNSC) with normal karyotype and high proliferation potential under XF conditions. Interestingly, adherent hNSC derived from H9, Shef4, and iPS6-9 differentiated into significant numbers of O4+ oligoprogenitors (approximately 20-30%) with robust proliferation; however, very few GalC+ cells were observed (approximately 2-4%), indicative of early oligodendrocytic lineage commitment. Overall, these data demonstrate the transition of multiple hESC and hiPSC lines onto XF substrate and media conditions, and a reproducible neuralization method that generated neural derivatives with multipotent cell fate potential and normal karyotype.

Scientific Abstract:
Human embryonic stem cells (hESC) and induced pluripotent stem cells (hiPSC) can differentiate into many cell types and are important for regenerative medicine; however, further work is needed to reliably differentiate hESC and hiPSC into neural-restricted multipotent derivatives or specialized cell types under conditions that are free from animal products. Toward this goal, we tested the transition of hESC and hiPSC lines onto xeno-free (XF) / feeder-free conditions and evaluated XF substrate preference, pluripotency, and karyotype. Critically, XF transitioned H9 hESC, Shef4 hESC, and iPS6-9 retained pluripotency (Oct-4 and NANOG), proliferation (MKI67 and PCNA), and normal karyotype. Subsequently, XF transitioned hESC and hiPSC were induced with epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) to generate neuralized spheres containing primitive neural precursors, which could differentiate into astrocytes and neurons, but not oligoprogenitors. Further neuralization of spheres via LIF supplementation and attachment selection on CELLstart substrate generated adherent human neural stem cells (hNSC) with normal karyotype and high proliferation potential under XF conditions. Interestingly, adherent hNSC derived from H9, Shef4, and iPS6-9 differentiated into significant numbers of O4+ oligoprogenitors (approximately 20-30%) with robust proliferation; however, very few GalC+ cells were observed (approximately 2-4%), indicative of early oligodendrocytic lineage commitment. Overall, these data demonstrate the transition of multiple hESC and hiPSC lines onto XF substrate and media conditions, and a reproducible neuralization method that generated neural derivatives with multipotent cell fate potential and normal karyotype. J. Comp. Neurol., 2014. (c) 2014 Wiley Periodicals, Inc.

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